

# The Microbes of the Sulphur Works Area: A Field Guide

## What this Guide is Meant to Do

This guide is meant to give the reader a short introduction to the types of microorganisms we will see in our walk to the thermal features in the Sulphur Works area of Lassen Volcanic National Park (see Figure 1: Map of Sulphur Works, and Figure 2). Why on Earth would we be interested in the microbes when there is such nice scenery to take in (answer: read “Appendix I: Hey, Why Does NASA Care About Microbes in Lassen Volcanic National Park, Anyway?”). We will discuss some of the more common microbes you are likely to see (without a microscope, even!) as well as some of the factors that control their distribution. Of course, this is still an active area of research for microbial ecologists (we haven’t figured it all out yet). Nevertheless, there are certain general rules of thumb at work, which can be used to understand why certain microbes are more dominant in some locations than others. Take this guide along on our field trip as a source of information about which microbes you are likely to see on our whirlwind tour.

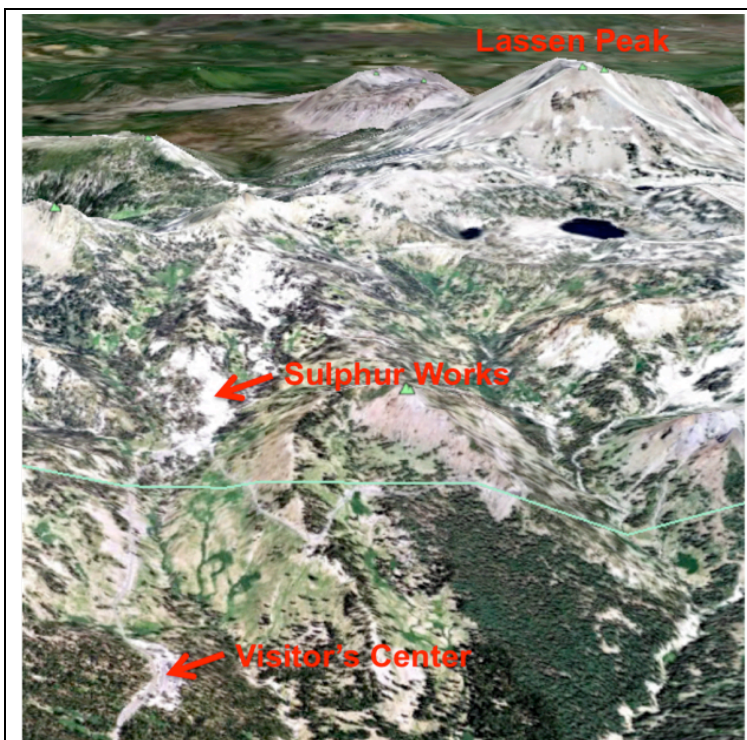


Figure 1. Map of Sulphur Works at Lassen Volcanic National Park

## Introduction

The colors of the snow along the trail we’ll be hiking today, as well as the edges of many of the thermal features, are produced by the growth of many different kinds of microorganisms. There can be just a few microbial cells in pockets of water in the snow or on rocks in the thermal areas, or thick accumulations of biomass known as microbial mats. In this guide, we will discuss why microbiologists and microbial ecologists are so interested in microbes in ice and in thermal features as well as some the microbes found in the Sulphur Works area, and how you might be able to recognize them.

Microorganisms are the earliest forms of life on our planet, dating back to some 3.5 billion years ago. The larger plants and animals around you, and with which you are probably a lot more familiar, are much more recent, dating back “only” a half billion years. That means that microbes were the only living things on Earth for a very long time. With the evolution of animals came the evolution of radulas, teeth, mandibles, claws and other mechanisms by which animals ingest (and subsequently digest)

microbes. It is generally thought that the evolution of grazing animals has a lot to do with the fact that microbes are much more visible in so-called “extreme environments.” Animals are not as well adapted to environments that feature such “extreme conditions” as very hot, and even hot and acidic water (hot springs), very cold water (polar lakes and ice), very salty water (soda and saline lakes and lagoons), and even places where there is very little water (deserts). Therefore, microbes in these environments are able to accumulate much greater biomass, enough biomass, in fact, to attract the attention of not only microbial ecologists, but tourists as well.



Figure 2. Sulphur Works viewed from the Ridge Lakes trail. Notice how the snow is melted off the geothermally heated ground (see the steam vent?). Everywhere else the snow depth is approximately 14 feet. That's a lot of heat! Photo taken 23 May 2011.

There are thousands and thousands of different kinds of microbes in the Sulphur Works area of Lassen Volcanic National Park. Many of these microbes are competing with each other for the same kinds of resources. These resources include reduced (energy containing) chemical compounds in the water, light, nutrients, even the very substrate upon which the microbes live. Microbes that are able (or better able) to utilize the available resources, and survive and grow better than all of their competitors in a given environment, will become more dominant (and more visible to us) in that environment. Over very long periods of time, the microorganisms become better and better “adapted” to that environment. So... the microorganisms that we see in any given thermal feature are there because they are “better” at living under those conditions than are their competitors. In Lassen Volcanic National Park, we can see the results of millions of years of adaptation and evolution in a single day's tour. We can then ask questions, (and even get some answers!), as to why certain kinds of microbes are found in certain kinds of places. For example: Why is it that Organism X (for example) is not in this hot spring?, Why are there so many of Organism Y here?, and so on.

## Classification of Microbes

Generally speaking, microbial ecologists classify organisms both phylogenetically (who they are related to) and metabolically (what they eat or obtain their energy from, read: “Appendix II: What's in a (Ridiculously Long) Name?”). Phylogenetically, the microbes in Lassen Volcanic National Park are classifiable into one of three “Domains of Life”, the Archaea, the Bacteria, and the Eukarya (read: “Appendix III: The “Big Tree of Life” for more information on this system). Another type of life, viruses, do not fall into any of these three domains, but are extremely numerous. A scientist from Portland State University, Dr. Ken Stedman, is studying the viruses in Sulphur Works and Boiling Springs Lake (see the brochure “Life in Boiling Acid”). Notable in the areas that we are visiting today are algae and chemolithotrophic bacteria. In other areas of the park, we may also see cyanobacteria. Algae and

Cyanobacteria, (or “Blue Green Algae” as they were previously known), are photosynthetic. They perform the same kind of photosynthesis (oxygen-producing, or “oxygenic” photosynthesis) as do the plants with which you are probably more familiar (trees, flowers, crops). In fact, cyanobacteria “invented” oxygenic photosynthesis; they were the first organisms to perform this process (read: “Appendix V: Oxygenic Photosynthesis and Pigments” for more information on this).

## Microbes You Can Expect to See at Sulphur Works

### *Snow Algae*

Help! Nathan

#### *Microbes of the Thermal Features*

The water emanating from many (but not all) thermal features in the Sulphur Works area is quite acidic due to the production of sulfuric acid from sulfur compounds dissolving into the water there. In fact, some researchers refer to the environment as Acid Sulfate springs to highlight the importance of the ionic composition of the water to the microbial processes that can occur there. Minerals dissolved in the water are a primary source of energy for many of the microbes found in this environment (see: Appendix IV: Oxidation – Reduction Explained, or...How to Talk to a Chemist if you Really, Really Have To for a rather detailed explanation of how this works).

The acidic pH of the water selects for a very different combination of hot spring microbes. In fact, many of the colors that you see in the springs at Sulphur Works are due not to cyanobacteria, as at many other thermal springs, such as those in Yellowstone National Park, but rather microbes that are using various minerals present in the water as sources of energy (chemolithoautotrophs), and chloroplast-containing eukaryotic algae (which are photolithoautotrophs). The yellowish color that you see in patches near steam vents is elemental sulfur (hence the name Sulphur Works!) left over from microbial oxidation and/or out-gassing of hydrogen sulfide. The rust color in the stream channel near the road (see Figure 4) is just that, iron oxide left over from the oxidation of iron dissolved in the water. In a hydrothermal feature along the Ridge Lakes trail, the lime green-colored microbial mat is composed of acidophilic (“acid loving”) eukaryotic algae *Cyanidia*. Despite their bright green color, these algae are actually classified with the red algae, which may be more familiar to you from trips to rocky sea coasts (or as the seaweed wrapper around many types of sushi). Note the very distinctive green color; it is a color of green that looks much more like “lime green” than the more blue-green color of cyanobacteria, although in winter, with low light, the color is a deeper blue green.



Figure 3. Snow algae.





Boiling mud pot on the side of the road at Sulphur Works during the summer. Note the level of liquid.



This is the same boiling mud pot during the winter that has become swollen with rain and melted snow.



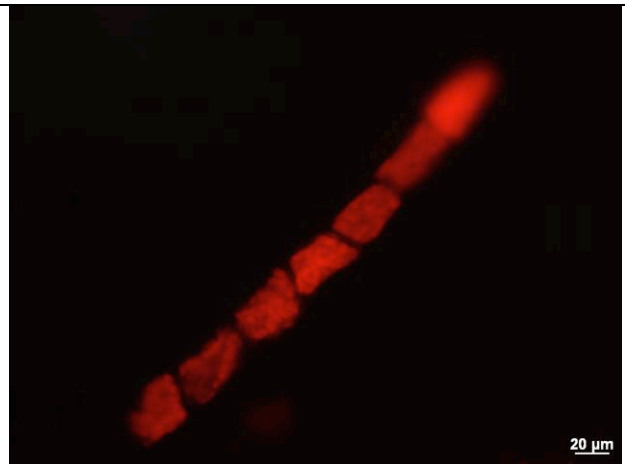
Lassen Astrobiology Student Interns measuring the temperature and pH of a Sulphur Works hydrothermal feature near the road. Note all of the rusty iron deposits on the rocks.



At the same site, measuring the pH of a microbial mat made up of acid-tolerant algae. See the oxygen bubbles on top of the mat from the algae performing photosynthesis. This oxygen contributes to the formation of the rusty iron deposits by oxidizing the iron in solution in the stream.



The algae from the microbial mat above viewed under a microscope. Note the chloroplasts, which contain the green pigment Chlorophyll *a*.



When ultraviolet (UV) light is shined on the algae, the Chlorophyll *a* is excited to a higher energy state and fluoresces red.

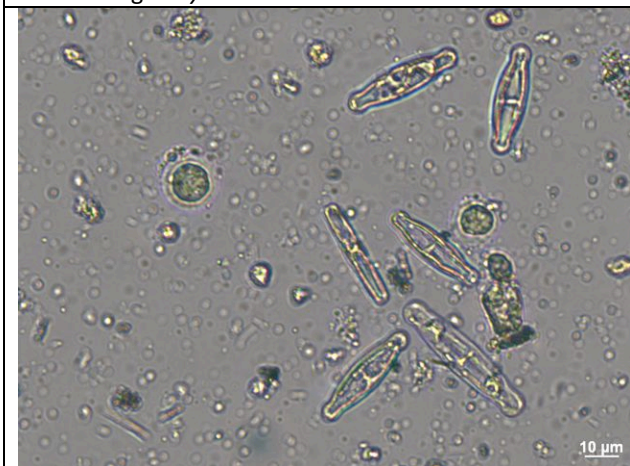




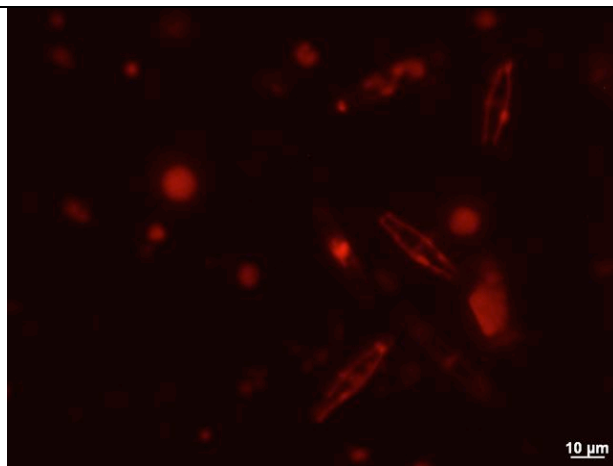
Lassen Astrobiology Student Interns measuring the temperature and pH of a hydrothermal feature at Sulphur Works. The lime green-colored microbial mat covering the bottom of the pool contains the acid-tolerant algae *Cyanidia*.



The students collecting water samples at the same site during the winter. The deep Sierra snowpack presents some challenging sampling issues that have to be solved with good old-fashioned ingenuity!



Sample of the apple green-colored microbial mat from the site in the photo above. The coccoidal cells are *Cyanidia* and the flying saucer-shaped organisms are diatoms with a shell made out of silica.



Same image as on the left, but the cells were illuminated with UV light, which makes their green pigments (Chlorophyll *a*) fluoresce red.

Figure 4. Hydrothermal habitats at Sulphur Works and their microbial inhabitants.

## Web Based References

### STEP Course Materials

<http://microbes.arc.nasa.gov/STEP>

### Microbes@NASA

<http://microbes.arc.nasa.gov/>

### Microbial Life Educational Resources

<http://serc.carleton.edu/microbelife/>

### Micro\*scope

<http://starcentral.mbl.edu/microscope/portal.php>

### Microbes at Lassen's Boiling Springs Lake

[http://web.pdx.edu/~kstedman/BSL/BSL\\_MO/The\\_BSL\\_MO.html](http://web.pdx.edu/~kstedman/BSL/BSL_MO/The_BSL_MO.html)

## Appendix I

### **Hey, Why Does NASA Care About Microbes in Lassen Volcanic National Park, Anyway?**

We're glad you asked. NASA is looking for life "out there" and... we haven't yet found it. Microbes were the first life on Earth, and were, in fact, the only life on Earth for well over half the time life has been here. Microbes have relatively simple environmental and nutritional requirements and have been found nearly everywhere, including so-called "extreme environments". Our atmospheric composition is the product of microbial metabolism (see the "Oxygenic Photosynthesis" box). In short, our only example of a planet with life on it is Earth, and Earth is literally run by microbes. If we use Earth as an example of life (and we have to, right?) microbial life may be a good bet for what to look for on other planets. Besides, if life on other existed, and was as "advanced" as we are, it (they?) would have signaled us by now, right?

Life on Earth, and very likely life elsewhere (if it exists), requires the presence of water and accessible carbon. NASA has developed a search strategy called "follow the water" in its search for life (probably microbial) on other planetary bodies in our solar system. While today liquid water is rendered unstable at the Martian equator by the low temperature and pressure, it was not always so. Indeed, early in Mars' history, it was very much a habitable planet like our Earth. This was confirmed by the Spirit and Opportunity Rovers, which found evidence indicating that liquid water once flowed freely on the surface of the planet. The presence of volcanoes also indicates that Mars was once volcanically active (much like Lassen Volcanic National Park), and that warm, wet subsurface conditions and warm surface springs were probably niches for early life to flourish.

NASA is interested in studying the microbes living in the hydrothermal systems at Lassen as an "analog" for the types of environments that may have once existed on a young Mars. Not only is NASA interested in how the microbes or extremophiles survive, but also how they become fossilized. It's likely that there are no living microbes on Mars today due to the inhospitable conditions, so instead NASA is searching for microbial fossils to prove that life once existed. In fact, the upcoming Mars Science Laboratory mission launching in November 2011 has a payload of instruments designed to look for evidence of fossilized microbes in mineral deposits.

Hydrothermal systems on Earth concentrate minerals (they precipitate out of solution as the water in which they were previously dissolved cools). As minerals precipitate, they can entomb live organisms (as well as some of the chemical compounds, "biomarkers", that they were made of). Some Lassen silica deposits contain evidence (chemical and morphological) of microbial life. If life ever existed on Mars, it might have left the same kinds of "trace fossils" of microbial activity in hydrothermal areas. Finally, the gases produced by microbes in thermal (as well as many other) environments are released into the atmosphere. NASA's present search strategy to look for life on extrasolar planets (too far to be reached by manned or unmanned vehicles) is to look into the atmospheres of those extrasolar planets for the "gases of life". We do research on microbial communities to find out how, where, and under which conditions, these gases are produced and released into the atmosphere where planned missions such as Terrestrial Planet Finder can detect them.



## Appendix II

### What's in a (Ridiculously Long) Name?

Microbial ecologists, biogeochemists, microbiologists, and that guy at the cocktail party who wasn't talking to anyone use a seemingly endless (both the number of words and their lengths) series of words to refer to the metabolisms of microorganisms. The term that we use conveys information about how the microorganism obtains the energy, electrons, and the "building blocks" (in the case the "building blocks", we refer only to the source of carbon), in the following order:

Energy Source\_ElectronDonor\_CarbonSource\_troph"

For a word like:

Chemolithoautotroph

"chemo" means that the energy is coming from "chemicals" (as opposed to light)

"litho" means that electrons are coming from inorganic sources, from the Greek for "rock"

"auto" refers to the fact that it uses inorganic (not organic) carbon, (as opposed to "hetero", which would denote carbon coming from "other" organisms)

"troph" again, what the organism eats, from the Greek for "feeder" or "nutrition" or "eater"

So... a chemolithoautotroph uses the energy and electrons from inorganic compounds, as well as carbon from an inorganic source, for nutrition. An example would be a sulfide oxidizer, which uses using hydrogen sulfide, and carbon dioxide to make food its own food (i.e., new cell material).

Now try:

photolithoautotroph

A photoautotroph uses light (photo), and electrons from inorganic sources (litho) to make food using inorganic carbon (auto) sources. Most of the plants that we know are photolithoautotrophs.

How about:

chemoorganoheterotroph

Think about the guy at the cocktail party. He is using energy (chemo), electrons (organo) and carbon (hetero), all from those little cocktail wieners. He obviously does not have a lot going on. No wonder no one will talk to him.

Why bother with all these long names? Firstly, they are great to have for breaking the ice at cocktail parties. Secondly, the metabolisms of the organisms is important to know and to convey; it has a lot to do with where they are found, their role in the environment, and their role in the complex microbial communities where they live. It would, of course, be very nice if the phylogenetic and metabolic classification schemes divided organisms up in a similar manner. It *is* the case that very closely related organisms are more likely to have similar metabolic strategies than distantly related ones. However, many different types of organisms, not closely related at all, can also perform very similar metabolic functions. Similarly, many organisms that are related closely to each other can do very different things for a living.

## **Appendix III**

### **The “Big Tree of Life”**

Over the years, biologists have used various schemes to attempt to place the amazing diversity of life into categories that could be studied, understood, and most importantly, stuffed and/or dried and placed into separate drawers at the Natural History Museum (where they belonged). The most fundamental division, used at least until around 1970, was the basic division between Plants (Plantae) and Animal (Animalia). At about that time Roger Whittaker proposed that life actually should be divided into five Kingdoms (Monera, Protista, Fungi, Plantae, and Animalia) and this scheme is still used by many of our textbooks.

However, in 1977, Carl Woese, who had figured out a way to classify organisms using the sequence of nucleic acids present in a gene that all organisms contain (the gene for ribosomal RNA), found that Whittaker’s Kingdom Monera actually contained two groups of organisms which were wildly different from each other. He called the new group of organisms the Archaea. In fact, Carl Woese’s new analysis showed that the five kingdom system of classification needed to be thrown out entirely, in favor of a new system in which the new group formed one of three “Domains” of Life, the other two being the Bacteria and the Eucarya.

There are big differences between the systems. Two of the three new Domains of life in the new system (Archaea and Bacteria) had previously been “lumped” into one kingdom in the old system (Monera), whereas four of the five Kingdoms in the old system (Protista, Fungi, Plantae, and Animalia) had been used to “split” organisms which are now placed into one Domain (Eucarya). Plants and Animals, once recognized as the most basic difference between organisms, turn out to actually be very similar to each other (their ribosomal genes are very similar).

Because the ribosomal RNA gene is considered so basic (and important) to the functioning of cells composing organisms in all organisms, the new system is considered to be a much more reliable indicator of relationships among organisms and the new classification system is almost universally adopted. The so-called “Big Tree of Life” now has three main branches, the Archaea, the Bacteria, and the Eucarya; the branches indicate lines of descent (which organisms came from which) with the lengths of the branches indicating how closely they are related. A few interesting features of the Big Tree, 1) almost everything around you that is big enough to see (plants, animals, fungi) is very closely related, 2) most of the diversity of life (most branches of the tree) are too small for you to see (they are microbial), and 3) we (as members of the Domain Eucarya) are more closely related to Archaea than Bacteria and Archaea are related to each other (even though they are both microbial and previously lumped into the Kingdom Monera). Another term that was previously used to describe both Bacteria and Eucarya, “procaryote”, is also being re-evaluated. Because of the huge differences that exist between these two groups, some scientists, (Norm Pace prominent among them), are arguing that the term “procaryote” should no longer be used.



## **Appendix IV**

### **Oxidation – Reduction Explained, or...**

#### **How to Talk to a Chemist if you Really, Really Have To**

Chemists will tell you, if you can stay awake long enough, that life can basically be described as something that mediates the flow of electrons from one element in the periodic table to another element in the periodic table (it is all about the periodic table to these folks). Every element in the periodic table can be described in terms of its tendency to either give up (“donate”) or take up (“accept”) electrons. This tendency varies quite a bit, some elements really, really, really want to either take up or lose electrons, some elements really couldn’t care less. An important consequence of this is that any two elements, one of which “wants” to give up electrons, the other of which “wants” to accept electrons, have the potential to act as a battery, capable of creating a flow of electrons through a wire. To a chemist, life can be viewed as a kind of wire through which electrons flow from a donor to an acceptor. While this sometimes seems a rather unappreciative view of the miracle that is life, they are basically right (please don’t tell them, it’ll make their heads get even bigger). In the specific case of humans, the carbon in the food that we eat is the donor of electrons and the oxygen in the air that we breathe is the acceptor of the electrons. We refer to the process of losing electrons as an “oxidation” and the process of gaining electrons as a “reduction”. Therefore, in the process that starts with eating, we are oxidizing organic carbon (food) and reducing oxygen (to a chemist).

The water in which microbes live is a complex mixture of elements that have been dissolved away (“weathered”) from rocks. Some of these elements “want” electrons and some “want” to give up electrons; so there are many, many possible batteries dissolved in the water, the trick is to catch them and to use them. Over literally billions of years, microorganisms have developed ways (enzyme systems) to use some of the elements present in the water in which they live (or the surfaces upon which they grow) to generate a flow of electrons through themselves (which they use as a source of energy). As you can imagine, as with all things biological, the microorganisms that find the best pairs of elements to use as their batteries (the greatest “bounce per ounce”) get more energy, make more of themselves, and out-compete the microorganisms using less efficient batteries.

In places like Sulphur Works, where there are a lot of minerals dissolved in the water, and not as much photosynthesis, this competition for electron donors and acceptors is very important for structuring microbial communities. As element pairs making up the best battery are used up from the spring water, the next most efficient electron pairs are then used, usually by a different set of microorganisms. Because the minerals that are formed by the oxidation-reduction reactions are colored, this is observed as a color change from yellow (elemental sulfur from hydrogen sulfide) to the characteristic browns and reds of oxidized forms of iron and arsenic. At Sulphur Works, the best battery is made with hydrogen (as an electron donor) and oxygen (as an electron acceptor). NASA uses this same battery in space shuttle fuel cells. Next comes hydrogen sulfide (donor) and oxygen (acceptor). Then, when the hydrogen sulfide is used up (and converted to elemental sulfur), microbes use iron and arsenic compounds as electron donors (still using oxygen as an electron acceptor).

## **Appendix V**

### **Oxygenic Photosynthesis and Pigments**

The invention of oxygenic photosynthesis was arguably the most important metabolic invention in the history of the Earth. Before oxygenic photosynthesis, organisms were limited to sources of energy that leaked out of the Earth as chemical compounds. These chemical compounds (such as hydrogen sulfide, methane, and iron) provided the electrons (energy) that microorganisms used to make more of themselves. In oxygenic photosynthesis, light energy is used to obtain electrons from water, which is, of course, much more abundant than the chemicals dissolved in it. Using oxygenic photosynthesis, microorganisms are literally harnessing the power of the sun as a source of energy. The electrons from water are used to make food (sugar) from carbon dioxide. Molecular oxygen is released as a “waste product” of the reaction. The oxygen that you are currently enjoying originally came from cyanobacterial photosynthesis. Cyanobacteria, according to the theory of endosymbiosis, evolved into the chloroplasts of the more familiar plants.

Pigments are, by definition, colored (the color that we see is due to all the colors contained in white light that are not being absorbed by the pigment). Cyanobacteria (as well as other photosynthetic organism) use pigments both to absorb light energy for use in photosynthesis, and to protect themselves from harmful ultraviolet radiation. So, the pigment complements that “work” for these phototrophs (are the most adaptive for any given environment) are comprised of a combination of pigments that absorb both the radiation that the organism uses to make its food, and the radiation that would be harmful, if it were to be absorbed by something more important than a pigment (like DNA or a protein). Pigments called carotenoids are used by many organisms both to supplement the light harvested by chlorophylls (the primary light harvesting pigment) and to protect against excessive light (carotenoids are responsible for the bright red color of the snow algae). Because different pigments are used by different phototrophs, and different phototrophs are adapted to different conditions, we see many different colors in thermal features.